DATA EVALUATION RECORD

METHYL BROMIDE

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 46665001

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by

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Task No. 123-2006

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6) OECD 426

PC CODE: 053201

DP BARCODE: D322563
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Methyl Bromide (99.9% a.i.)

SYNONYMS: None

CITATION: Beck, M.J. (2004) An inhalation developmental neurotoxicity study of methyl bromide in rats. WIL Research Laboratories, LLC., Ashland, Ohio. Study Number WIL-186039; December 3, 2004. MRID 46665001. Unpublished.

SPONSOR: The American Chemistry Council, Methyl Bromide Industry Panel, 1300 Wilson Boulevard, Arlington, VA 22209

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46665001), methyl bromide (99.9% a.i., lot # 4010PI136V) was administered by whole-body inhalation to 24 mated female Crl:CD®(SD)IGS BR rats/group at nominal concentrations of 0, 5, 25, or 50 ppm from gestation day (GD) 6-20 and females with selected pups from their litter were exposed on lactation days (LDs) 5-20. A Functional Observational Battery (FOB) was conducted on 12 dams/group on GDs 6 and 13 and LDs 10 and 21. On postnatal day (PND) 4, litters were standardized to eight pups; sexes were represented as equally as possible. Pups were weaned from their dam on PND 21 with no further exposure to the test material. Dams were sacrificed after weaning. A subset of 20 pups/sex/group was assigned to FOB, acoustic startle response, locomotor activity and learning and memory testing (PND 62). From this subset, 15 pups/sex/group were selected for neuropathological, morphometric, and brain weight evaluations on PND 72. A second subset of 20 pups/sex/group was selected for learning and memory (PND 26) and a third subset of 15 pups/sex/group was selected for neuropathological, morphometric, and brain weight evaluations on PND 21. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

One control female was sacrificed on gestation day 23 because of dystocia. All remaining animals survived to scheduled sacrifice. No clinical signs of toxicity were observed during the daily examinations, midway through the exposure, or 1-2 hours post-exposure. No treatment-related changes were noted during the FOB on any testing day. Maternal body weight and food consumption were not affected by treatment at any time during the study. No treatment-related effects were observed in reproductive parameters and gross necropsy was unremarkable.

No treatment-related effect on the mean number of pups born, mean live litter size, percentage of males per litter, or pup survival was observed. No treatment-related abnormalities were noted post-weaning during weekly physical examination.

Pup body weight was similar between the treated and control groups on PNDs 1-11. On PNDs 13-21, mean body weight was significantly ($p \leq 0.05$ or 0.01) decreased in the high-concentration female offspring (90-92% of control value) and was slightly (n.s.) or significantly decreased in the high-concentration male offspring (92-94% of control value). Mean body weight gain was significantly ($p \leq 0.05$ or 0.01) decreased in the high-concentration females (82-89% of control value) during PNDs 7-11 and 13-17 and for PND 4-21 (91% of controls). Mean weight gain was significantly ($p \leq 0.01$) decreased in the high-concentration males (83% of control value) during the PND 13-17 interval. The mid-concentration males and females also had reduced body weight gain (87-88% of control value) during the PND 13-17 interval. Post-weaning, absolute body weight of the high-concentration group was significantly ($p \leq 0.05$ or 0.01) less than that of controls through PND 56 for males (92-95% of controls) and PND 42 for females (91-95% of controls). Thereafter until study termination on PND 72, body weight was comparable between the treated and control groups in both sexes. Weight gain by the high-concentration males and females was significantly ($p \leq 0.01$) less than that of the controls during the PND 28-35 interval. Body weight gain was similar between the treated and control groups for all intervals after PND 35. The average age of onset of preputial separation in males was significantly ($p \leq 0.05$) delayed by 1.4 days in the high-concentration group compared with the controls. The average age of onset of vaginal opening was for the high-concentration females was significantly ($p \leq 0.01$) delayed by 1.6 days compared with the controls. Body weight in the treated males and females was similar to that of the control group at the time of acquisition.

No treatment-related FOB changes were observed in males or females on any testing day. Auditory startle response and learning and memory were not affected by treatment. No statistically significant difference in total activity or ambulatory activity was found between the treated and control groups on any testing day. However, on PND 21 total and ambulatory activities of high-concentration males were 60% and 54%, respectively, of the control levels, and for high-concentration females were 63% and 60%, respectively, of the control levels. Mid-concentration females had total and ambulatory activities 76% and 68%, respectively, of the control levels on PND 21. In these treated groups, the level of activity was reduced throughout the testing interval although the pattern of habituation was not affected.

Brain weight, gross necropsy, and microscopic findings were similar between the treated and control groups. On PND 21, high-concentration males had significantly smaller brain width (14.7 mm vs 15.1mm for controls; $p \leq 0.05$). No other treatment-related differences in any brain morphometric measurement were noted between treated and control groups for either sex at any time point.

The maternal systemic and neurotoxicity LOAEL for methyl bromide in rats is not identified and the maternal NOAEL is ≥50 ppm.

The offspring systemic and neurotoxicity LOAEL for methyl bromide in rats is 25 ppm based on decreased body weight gain in males and females and decreased motor activity in females. The offspring NOAEL is 5 ppm.

This study is classified **Acceptable/Guideline** and does satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426. It is noted that adequate positive control studies have been submitted to demonstrate proficiency of the testing facility only for FOB, motor activity, and auditory startle tests in young adult rats. Adequate positive control data have not been submitted for learning and memory or neuropathology and morphometrics.

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Methyl bromide

Description:	liquified gas
Lot #:	4010PI136V
Purity:	99.9 % a.i.
Compound Stability:	expiry date two years from opening the container
CAS # of TGAI:	74-83-9
Structure:	CH_3 - Br

2. Vehicle: The test article was mixed with filtered room air.

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD®(SD)	
Age at mating:	not given	
Wt. at study initiation:	233-280 g (GD 6)	
Source:	Charles River Laboratories, Portage, MI	
Housing:	Females were individually housed in plastic maternity cages with nesting material. After weaning on PND 21, the offspring were housed by sex with litter mates in plastic cages with nesting material through PND 28. On PND 28, offspring were individually housed in wire-mesh cages and remained in these cages until euthanasia.	
Diet:	PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002, *ad libitum*	
Water:	Reverse osmosis drinking water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	50±20%
	Air changes:	10/hour
	Photoperiod:	11 hrs dark/13 hrs light
Acclimation period:	none	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: December 1, 2004; End: March 26, 2005

2. **Study schedule:** Mated female rats (24/exposure group) were exposed to the test material by whole-body inhalation for 6 hr/day from gestation days (GDs) 6-20 and females with selected pups from their litter were exposed on lactation days (LDs) 5-20. A Functional Observational Battery (FOB) was conducted on 12 dams/group on GDs 6 and 13 and LDs 10 and 21. On postnatal day (PND) 4, litters were standardized to eight pups; sexes were represented as equally as possible. Pups were weaned from their dam on PND 21 with no further exposure to the test material. Dams were sacrificed after weaning. A subset of 20 pups/sex/group was assigned to FOB, acoustic startle response, locomotor activity and learning and memory testing (PND 62). From this subset, 15 pups/sex/group were selected for neuropathological, morphometric, and brain weight evaluations on PND 72. A second subset of 20 pups/sex/group was selected for learning and memory (PND 26) and a third subset of 15 pups/sex/group was selected for neuropathological, morphometric, and brain weight evaluations on PND 21.

3. **Mating procedure:** Females were received time-mated from the supplier.

4. **Animal assignment:** Mated females were assigned to groups using a computerized randomization procedure that assigned animals based on stratification of the GD 3 or 4 body weight into a block design, as shown in Table 1.

TABLE 1. Study design				
Experimental parameter	**Exposure concentration (ppm)**			
	0	**5**	**25**	**50**
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	24	24	24	24
FOB (GDs 6 and 13)	12	12	12	12
FOB (LDs 10 and 21)	12	12	12	12
Offspring				
	No. of offspring assigned			
Subset A - FOB (PNDs 21, 35, 45, and 60); Acoustic startle response (PND 20 and 60); Locomotor activity (PNDs 13, 17, 21 and 61); Learning and memory (PND 62).	20/sex	20/sex	20/sex	20/sex
Subset A - neuropathological, morphometric and brain weight evaluations (PND 72)	15/sex	15/sex	15/sex	15/sex
Subset B - Learning and memory (PND 26)	20/sex	20/sex	20/sex	20/sex
Subset C- Neuropathological, morphometric and brain weight evaluations (PND 21)	15/sex	15/sex	15/sex	15/sex

5. **Dose selection rationale:** Exposure concentrations were chosen based on the results from a range-finding developmental neurotoxicity study; the results of the range-finding study were included as an appendix with the main study. Groups of 10 female rats with litters were exposed for 6 hr/day to 0, 3, 10, or 30/90 ppm on lactation days 5-10. Due to lack of any signs of toxicity the 30 ppm concentration was increased to 90 ppm after 1-3 days of exposure. All dams and pups were sacrificed on lactation day 11 and subjected to gross examination. No clinical signs of toxicity were observed in any animal, and maternal body weight and offspring growth were not affected at any concentration. Based on these results and the results of previous studies (no further details), concentrations for the current study were chosen as 0, 5, 25, and 50 ppm.

6. **Animal exposure:** Pregnant rats were exposed to the test material by whole-body inhalation for 6 hr/day from GDs 6-20 and females with selected pups from their litter were exposed on LDs 5-20. After post-natal day (PND) 20, offspring were not exposed. Exposure chambers were 2.0 m^2 stainless steel and glass chambers. The supply air was provided from a HEPA- and charcoal-filtered, temperature- and humidity-controlled source. During exposure, females were housed in a suspended cage battery during the gestation phase and females with litter were housed in 4.7 L annealed-glass cylinders during the lactation phase. Food and water were withheld during exposure. However, a water substitute (Transgel®) was provided during the lactation phase to reduce stress on nursing dams.

7. **Atmosphere generation:** In each chamber, the atmosphere was generated from a Tedlar® gas sampling bag filled daily with the test article and used as a generator reservoir. The gas bag was placed in a sealed polycarbonate generation box during the exposure period. To permit

delivery of the methyl bromide gas to the exposure system, the outlet of the gas bag was connected to a bulkhead fitting mounted through the wall of the generation box and connected to a metering flowmeter. To provide the force to push the test article from the gas bag, the generation box was pressurized via a port connected to the facility compressed air system. Test article was delivered to the chamber inlet pipe, where the concentration was reduced to the desired exposure level by mixing with the chamber ventilation air. Chamber temperature, relative humidity, and ventilation rate were monitored continuously during exposure. During the gestation period, temperature was 22-25 °C and humidity was 35-59% with 12-15 air changes per hour. During the lactation period, temperature was 21-25 °C and humidity was 35-61% with 33-38 air changes per hour. Oxygen content of all chambers was above 19%.

Concentrations within each chamber were measured ten times during each daily exposure period using a gas chromatograph with a flame ionization detector. Nominal exposure concentrations were determined by weighing the test article containers prior to and following each daily exposure period, then calculating the concentration from the weight difference and the total volume of air passed through each chamber during the daily exposure. Homogeneity was evaluated during method development, prior to animal exposures, from samples taken from ten test locations and a reference location. For each test location, the measured concentration was calculated as a percent difference from the reference location.

Results:

Homogeneity analysis: Mean concentrations of samples from the test locations varied by less than 4% from the reference location.

Particle size determination: This was not measured since the test article is a gas and no aerosol is expected.

Concentration analysis: Absence of test article was confirmed in the control chambers. Overall mean nominal concentrations of the 5-, 25-, and 50-ppm chambers were 5.2, 21, and 42 ppm, respectively, during gestation and 4.5, 22, and 43 ppm, respectively, during lactation. Overall mean analytical concentrations of the 5-, 25-, and 50-ppm chambers were 5.0, 25.1, and 50.3 ppm, respectively, during gestation and 5.1, 25.1, and 50.4 ppm, respectively, during lactation.

The analytical data indicated that the chamber atmospheres were homogeneous and that the difference between nominal and analytical concentrations was acceptable.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Females were observed twice daily for mortality, moribundity, and clinical signs of toxicity; observations were conducted at the approximate midpoint of exposure and approximately 1-2 hours after completion of exposure. Clinical signs were recorded twice weekly for each individual.

Up to 12 females in each group were evaluated in a functional observational battery (FOB) at least twice during the gestation period (days 6 and 13) and lactation period (days 10 and 21). The examiner was unaware of the animal's group assignment. No details were provided on the arena size or examination procedures; scoring criteria were listed in the results tables. Pupillary function was not measured. Animals were evaluated for ease of removal from the cage, ease of in-hand handling, and the following functional observations outside the home cage.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe (note: scoring criteria not given) 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded and food consumption was measured on GDs 3 or 4 (body weight only), 6, 9, 12, 15, and 20 and on lactation days 1, 4, 7, 10, 13, 17, and 21. Food consumption was reported as g/animal/day and g/kg/day. All females were allowed to deliver naturally and rear their young until weaning on lactation day 21.

b. Offspring:

1) **Litter observations:** The day of initiation of parturition was designated as PND 0. When parturition was complete, the number of stillborn and live pups in each litter was recorded and the pups were examined for gross malformations. Pups were individually sexed on PNDs 0, 4, 11, and 21. All litters were observed once daily for survival and clinical signs of toxicity. A detailed physical examination of each pups was conducted on PNDs 1, 4, 7, 11, 13, 17, and 21 and at weekly intervals, thereafter, until necropsy.

   On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, when possible). Culled pups were weighed, euthanized on PND 4, and discarded. If a litter consisted of less than six pups or did not meet the sex ratio criteria (at least 3/sex), the litter was not used for neurobehavioral or neuropathological evaluation, but was maintained on study until scheduled euthanasia.

   Surviving pups were weighed on PNDs 1, 4, 7, 11, 13, 17 and 21 and weekly thereafter until necropsy (PND 72) and whenever they were removed from their cages for behavioral testing.

2) **Developmental landmarks:** Beginning on PND 35, male offspring were examined daily for preputial separation. Beginning on PND 25, female offspring were examined daily for vaginal patency. The age of onset and the offspring body weight at that time were recorded.

3) **Postweaning observations:** After weaning on PND 21, offspring were examined by cage-side observation once daily and a detailed weekly observation. Individual offspring body weight data were recorded weekly.

4) **Neurobehavioral evaluations:** Following litter standardization on PND 4, one male and one female from each litter (20 pups/sex/group) were assigned to the following neurobehavioral tests.

i) **Functional observational battery (FOB):** On PNDs 21, 35, 45, and 60, twenty offspring/sex/group were examined outside the home cage in a modified FOB assessment. The same animals were observed at each interval. The same parameters assessed in the maternal FOB were examined in offspring, with the addition of pupillary response, fore- and hind-limb grip strength, tail pinch response, hindlimb extension, and air righting reflex. No details were provided on the arena size or examination procedures; scoring criteria were listed in the results tables.

ii) **Motor activity testing:** Motor activity was evaluated in twenty pups/sex/dose on PNDs 13, 17, 21 and 61. The same animals were tested at each interval. Activity was measured using the SDI Photobeam Activity System in a room equipped with a white noise generation system set to operate at 70±10 dB. Data were collected in five-minute intervals for a test duration of 60 minutes. Data for ambulatory and total motor activity were recorded.

iii) **Auditory startle response:** Auditory startle response testing was performed on twenty offspring/sex/dose on PNDs 20 and 60 using the SR-Lab Startle Response System. The same animals were tested at each interval.

Testing was performed in a room equipped with a white-noise generation system set to operate at 70±10 dB. Each test session consisted of a five-minute acclimation period with a 65-dB broadband background white noise. The startle stimulus for each trial was a 115-dB mixed-frequency noise burst stimulus of approximately 20 milliseconds in duration. Responses were recorded during the first 100 milliseconds following the onset of the startle stimulus for each trial. Each session consisted of 50 trials with an eight-second intertrial interval. Startle response measurements included maximum response amplitude (V_{MAX}), average response amplitude (V_{AVE}) and latency to V_{MAX} (T_{MAX}), which were analyzed in five blocks of 10 trials each.

iv) **Learning and memory testing:** Learning and memory testing was performed on 20 offspring/sex/dose using a water-filled Biel maze. Animals were required to traverse the maze and escape by locating a platform hidden beneath the water surface. The amount of time required (maximum of three minutes) and the number of errors were recorded. An

error was defined as any instance when an animal deviated from the correct channel with all four feet.

The testing intervals were on PNDs 26 and 62; the same animals were not tested on each day. The testing intervals consisted of seven consecutive days. On day one, animals were placed in a straight channel opposite the escape platform and the time required for each animal to escape was recorded. Each animal was given four trials to assess swimming ability and motivation. On days 2 and 3, animals were allowed two trials per day for two consecutive days to solve the maze in path A. Animals were then allowed two trials per day for three consecutive days (days 4-6) to solve the maze in path B (reverse of path A). For each trial, animals were allowed three minutes to solve the maze. On the final day of testing, each animal was given two trials in path A. The minimum inter-trial interval was one hour.

2. **Postmortem observations**:

a. **Maternal animals:** All females were euthanized by carbon dioxide inhalation and subjected to gross examination. Females that did not deliver by post-mating day 25 were euthanized and the numbers of implantation sites and corpora lutea were recorded, if macroscopically evident. Uteri without macroscopic evidence of implantation were opened and placed in a 10% ammonium sulfide solution for detection of early implantation loss. Females with total litter loss were euthanized within 24 hours and the number of former implantation sites was recorded. For one female sacrificed moribund, the number of implantation sites and corpora lutea was recorded and late resorptions were examined. Tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as deemed necessary by gross findings and the carcass of each of these dams was discarded.

All females with viable pups on lactation day 21 were euthanized by carbon dioxide inhalation and subjected to gross necropsy. The number of former implantation sites was recorded. Nongravid uteri were placed in 10% ammonium sulfide. Tissues were preserved only as deemed necessary and the carcass of each dam was discarded.

b. **Offspring**: Offspring found dead or euthanized *in extremis* between birth and PND 4 were examined by fresh dissection. The stomach was examined for the presence of milk and the carcass was discarded. Pups culled on PND 4 were weighed, sacrificed by an intraperitoneal injection of sodium pentobarbital and discarded. Offspring not selected for neuropathology or behavioral evaluations were euthanized by carbon dioxide inhalation on PND 21 and subjected to gross necropsy. Tissues were retained only if deemed necessary by the gross findings, and the carcass was discarded. Pups scheduled for sacrifice on PND 72 and not allotted for neuropathology/brain weight measurement were euthanized by carbon dioxide inhalation and subjected to gross necropsy. Tissues were retained only if deemed necessary by the gross findings and the carcasses were discarded.

On PND 21, one male or female offspring from each litter (15/sex/group) was euthanized by carbon dioxide inhalation and perfused *in situ* with 4% paraformaldehyde/1.4%

glutaraldehyde according to the testing facility's standard operating procedure (T3-034). The whole brain (including olfactory bulbs) was removed, weighed and the size (length and width) recorded. Abnormal coloration or lesions of the brain and spinal cord were recorded. The brain from every animal was processed and embedded, however, only tissues from 10 control and 10 high-dose animals were examined microscopically. All brains were prepared for histopathological examination by embedding in paraffin, sectioning, and staining with hematoxylin and eosin. Sections from all major brain regions (olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, brainstem and cerebellum) were examined. At least two morphometric measurements were made on each of the neocortical, hippocampal, and cerebellar areas of the brain. Level 1 was a coronal section of the rostral cerebrum which included the height of the hemisphere and the verticle thickness of the cortex. Level 3 was a coronal section of the mid-cerebrum and included the radial thickness of the cortex, the vertical height between the hippocampal pyramidal neuron layers, the vertical height of the dentate hilus, and the length of the ventral limb of the dentate hilus. Level 5 was a mid-saggital section of the cerebellum and pons and included the vertical thickness of the pons and the base of lobule 9.

On PND 72, one male and one female from each litter (10/sex/group) were randomly selected from those involved in neurobehavioral testing and euthanized by carbon dioxide inhalation and perfused *in situ* with 4% paraformaldehyde/1.4% glutaraldehyde according to the testing facility's standard operating procedure (T3-034). The whole brain (including olfactory bulb) was removed, weighed and the size (width and length) recorded. The central and peripheral nervous tissues were preserved as described in the testing facility's standard operating procedure (T3-035) and embedded in paraffin or plastic, respectively. Tissues for all groups were processed and embedded, however, only control and high-dose tissues were examined microscopically. Tissues were prepared for histopathological examination by sectioning and staining with hematoxylin and eosin. Morphometric measurements were made on Levels 1, 3 and 5 of the brain, as described for PND 21. The following tissues from 10 control and 10 high-dose animals perfused *in situ* at study termination (PND 72) were examined microscopically:

X	CENTRAL NERVOUS SYSTEM			X	PERIPHERAL NERVOUS SYSTEM
	BRAIN				SCIATIC NERVE
X	Olfactory bulbs	X	Hippocampus	X	Cross section
X	Cerebral cortex	X	Basal ganglia	X	Longitudinal section
X	Central gray matter	X	Thalamus		OTHER
X	Cerebellum	X	Hypothalamus	X	Sural Nerve
X	Tectum	X	Cerebral peduncles	X	Tibial Nerve
X	Pons		Tegmenta	X	Peroneal Nerve
X	Medulla oblongata			X	Lumbar dorsal root ganglion (T_{13} - L_4)
	SPINAL CORD			X	Lumbar dorsal root fibers (T_{13} - L_4)
X	Cervical swelling (C_3 - C_7)			X	Lumbar ventral root fibers (T_{13} - L_4)
X	Lumbar swelling (T_{13} - L_4)			X	Cervical dorsal root ganglion (C_3 - C_7)
	OTHER			X	Cervical dorsal root fibers (C_3 - C_7)
	Gasserian ganglion			X	Cervical ventral root fibers (C_3 - C_7)
X	Trigeminal ganglion/nerves				
X	Optic nerve				
X	Eyes			X	Skeletal muscle (gastrocnemius)

D. DATA ANALYSIS:

1. **Statistical analyses:** Maternal and offspring body weight and body weight gain, maternal food consumption, litter weight, gestation length, implantation sites, number of pups and litter sizes, day and body weight at attainment of sexual maturation, brain weight and dimensions, and morphometric data were analyzed by a parametric one-way Analysis of Variance (ANOVA) to determine intergroup differences. If the ANOVA was significant, Dunnett's test was applied to compare the treated and control groups. Neuropathology data were analyzed using the Fisher's Exact Test. The Kruskal-Wallis nonparametric ANOVA test followed by Dunn's test was used to analyze mean litter proportions of pup viability and sex ratio.

   Locomotor activity, auditory startle, and continuous FOB data were analyzed with a repeated measure ANOVA (RANOVA). The analyses were conducted sequentially to first evaluate sex interactions and then to evaluate the exposure and time effects for both within a session and across sessions. For locomotor activity, factors in the model included treatment group, time interval, and the interaction of time and treatment. For auditory startle response, factors in the model included treatment group, trial block, and the interaction of block and treatment. For FOB data, factors in the model included treatment group, session, and the interaction of treatment and session. The monotonic dose response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dose levels. If the linear dose by time or trial interaction was significant, trend tests on treatment means were performed for each time interval; if not significant, the trend test was conducted across the pooled time intervals for the entire session only. Nonmonotonic dose responses were evaluated when no significant linear trends were detected, but the treatment and/or the interaction was significant. If the interaction was significant, the comparisons were conducted for each time interval or block; if only the treatment effect was significant, the comparisons were conducted across the pooled intervals or blocks for the entire session.

Analyses of learning and memory data were conducted independently for each phase. The time to escape was analyzed as described above with the term for trial in the model. If the distribution of the number of errors was heavily skewed toward zero, the analysis was conducted individually for each trial and sex, with the extended Mantel-Haenszel test.

For count, graded, dichotomous and descriptive FOB endpoints, each analysis endpoint was treated as categorical data with the extended Mantel-Haenszel test.

2. **Indices:**

   a. **Reproductive indices:** No reproductive indices were calculated.

   b. **Offspring viability indices:** The following litter and offspring indices were calculated from parturition and pup survival records.

   Mean live litter size = (Total no. viable pups on PND 0)/(No. litters with viable pups on PND 0)

   Birth index (%) = [$\sum$(Viable pups per litter on PND 0/No. pups born per litter) / No. litters per group] × 100

   Viability index (% per litter) = [$\sum$(Viable pups per litter on PND 4/No. pups born per litter) / No. litters per group] × 100

   Survival index (% per litter) = [$\sum$(Viable pups per litter at end of interval/Viable pups per litter at beginning of interval) / No. litters per group] × 100

3. **Positive and historical control data:** Historical control data submitted for neurobehavioral testing conducted at WIL Research Laboratories, LLC is listed in the table below. Data for males and females were included and the animals were the same strain of rat as used in the current DNT study. Data were presented as mean for each study and grand mean for all studies combined; minimum and maximum values were given for each endpoint. The method used in each of these tests was not described.

Historical control data for WIL Research Laboratories, LLC				
Test	**Age of rats**	**Number of studies**	**Date conducted**	**Route**
Startle response	PND 20 PND 21 PND 60	15 1 18	not stated	not stated
Biel maze	PND 22 PND 24 PND 26 PND 60 PND 62 PND 63 PND 70 PND 107	17 1 1 1 22 1 2 1	6/14/99 - 6/28/04	gavage, diet, drinking water, inhalation, intranasal
Motor activity	PND 13 PND 17 PND 21 PND 23 PND 60/61	6 6 19 2 21	not stated	not stated

Data submitted on CD; no MRID number assigned.

Historical control data for 19 morphometric measurements obtained in seven studies were also included for PND 11, 21, 22, and 72 males and females. However, these data were limited in that each measurement had only 0-4 data points. No information was given regarding tissue processing or the dates the studies were conducted. Another study, which included results for acoustic startle response on PND 20 pups and eleven morphometric measurements from PND 11 pups, was included with positive control data for the testing facility. However, this latter study was not dated, individual animal data were not included, startle response results were only presented graphically, and morphometry data were given as mean and standard deviation without minimum and maximum values.

Historical control data for reproductive endpoints for males and females, pup growth and survival, and offspring developmental landmarks were submitted separately. Data were included from 107 studies conducted between 6/17/96 and 2/3/04.

Positive control data were included for inter-observer reliability in the FOB (adult animals); FOB, auditory startle, locomotor activity, learning and memory, and neuropathology and morphometry (pups and adults); optimization of motor activity sessions (young adults); acoustic startle response (young adult); and motor activity (young adult). Details and a review of these studies were included in a previous DER for the DNT study with zeta-cypermethrin (MRID not assigned). In conclusion, WIL Research Laboratories, Inc. demonstrated proficiency only in conducting FOB, motor activity, and auditory startle tests in young adult rats. Data for learning and memory or neuropathology and morphometrics utilized in the DNT study were insufficient for evaluation of proficiency.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** One control female was sacrificed on gestation day 23 because of dystocia. All remaining animals survived to scheduled sacrifice. No clinical signs of toxicity were observed during the daily examinations, midway through the exposure, or 1-2 hours post-exposure. Hair loss was a common finding in treated and control animals.

   No treatment-related changes were noted during the FOB on any testing day. The only statistically significant difference found was a decrease in defecation count for the high-concentration group on GD 6.

2. **Body weight and food consumption**: Selected group mean body weight, body weight gain and food consumption values for pregnant and nursing dams are summarized in Table 2. No treatment-related differences were observed between the treated and control groups throughout the study.

TABLE 2. Selected mean (±SD) maternal body weight (g), body weight gain (g), and food consumption (g/animal/day)

Observations/study interval	Exposure concentration (ppm)			
	0	5	25	50
Gestation (n=23-24)				
Body wt. gestation day 6	252 ± 10.8	254 ± 11.2	254 ± 10.4	252 ± 8.5
Body wt. gestation day 9	269 ± 11.8	269 ± 11.7	268 ± 13.4	268 ± 9.2
Body wt. gestation day 12	290 ± 14.1	289 ± 12.5	289 ± 14.4	288 ± 10.7
Body wt. gestation day 15	311 ± 16.5	309 ± 12.7	308 ± 15.8	307 ± 10.9
Body wt. gestation day 20	376 ± 20.8	370 ± 17.1	371 ± 18.6	369 ± 14.9
Wt. gain gestation days 6-20	123 ± 14.0	117 ± 10.3	118 ± 12.0	117 ± 10.3
Food consumption gestation days 6-20	23 ± 1.6	23 ± 1.6	23 ± 1.7	23 ± 1.0
Lactation (n=24-25)				
Body wt. lactation day 1	287 ± 17.1	285 ± 12.6	288 ± 15.7	286 ± 13.0
Body wt. lactation day 4	312 ± 17.7	306 ± 12.7	312 ± 18.7	307 ± 13.4
Body wt. lactation day 7	318 ± 18.7	311 ± 15.1	317 ± 15.3	311 ± 11.3
Body wt. lactation day 13	343 ± 22.4	339 ± 14.8	341 ± 17.8	335 ± 15.0
Body wt. lactation day 21	328 ± 16.5	331 ± 16.8	332 ± 20.5	331 ± 16.9
Wt. gain lactation days 1-21	41 ± 15.5	46 ± 16.0	44 ± 16.1	45 ± 20.0
Food consumption lactation days 1-4	36 ± 4.7	34 ± 4.2	37 ± 5.6	36 ± 5.6
Food consumption lactation days 17-21	65 ± 5.9	67 ± 5.8	69 ± 7.0	69 ± 5.5

Data taken from Tables 7-9 and 11-13, pp. 222-225 and 227-229, MRID 46665001.

3. **Reproductive performance**: Results for the maternal animals are summarized in Table 3. The number of animals pregnant, mean gestation length, implantations/dam, and number of live litters were not affected by treatment. The low-concentration group had significantly fewer mean implantation sites than the control group. One control dam was sacrificed *in extremis* on GD 23 with dystocia; necropsy revealed 12 late resorptions. In the mid-concentration group, one dam was not gravid and one dam had complete litter loss on lactation day 0.

TABLE 3. Reproductive performance

Observation	Exposure concentration (ppm)			
	0	5	25	50
Number mated (pregnant)	24 (24)	24 (24)	24 (23)	24 (24)
Pregnancy rate (%)	100.0	100.0	95.8	100.0
Intercurrent deaths	1	0	0	0
Number of live litters	23	24	22	24
Implantation sites/dam (mean±SD)	13.5 ± 1.44	12.0** ± 1.84	12.7 ± 1.58	13.0 ± 1.37
Mean (±SD) gestation length (days)	21.6 ± 0.51	21.5 ± 0.51	21.7 ± 0.45	21.5 ± 0.51

Data taken from Tables 1, 15, and 20, pp. 154, 231, and 236, respectively, MRID 46665001.
Significantly different from control: **$p \leq 0.01$.

4. **Maternal postmortem results:** No treatment-related gross lesions were observed in any animal.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) of pups during lactation are summarized in Table 4. No treatment-related effect on the mean number of pups born, mean live litter size, percentage of males per litter, or pup survival was observed. The low-concentration group had significantly fewer pups born resulting in a smaller mean live litter size on PND 0 compared with that of the controls. In the mid-concentration group, the significantly smaller live litter size on PND 0 was due to complete litter loss by one dam.

   The incidence of clinical signs during lactation was comparable between the treated and control groups. No treatment-related abnormalities were noted post-weaning during weekly physical examination.

TABLE 4. Mean (±SD) litter size and viability				
Observation	Exposure concentration (ppm)			
	0	5	25	50
Number of live litters	23	24	23	24
Mean no. pups born	12.8 ± 1.77	11.3** ± 1.76	11.9 ± 1.79	12.2 ± 1.34
Mean live litter size (PND 0)	12.8 ± 1.78	11.3* ± 1.73	11.3* ± 3.04	12.1 ± 1.26
Total litter loss/sacrificed	0	0	1	0
Sex ratio (% males/litter)	49.8 ± 16.49	49.7 ± 17.16	49.4 ± 12.86	50.1 ± 18.21
Live Birth Index (PND 0; % per litter)	99.7 ± 1.60	99.7 ± 1.57	95.7 ± 20.85	99.7 ± 1.36
Viability Index [PNDs 1-4(precull); % per litter]	99.7 ± 1.30	99.7 ± 1.70	99.5 ± 2.13	100 ± 0.00
Lactation Index [PNDs 4(post-cull)-21; % per litter]	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Data taken from Tables 21 and 22, pp. 237 and 238-239, respectively, MRID 46665001.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

2. **Body weight:** Selected mean preweaning pup body weight and body weight gain data are presented in Table 5. Pup body weight was similar between the treated and control groups on PNDs 1-11. On PNDs 13-21, mean body weight was significantly decreased in the high-concentration female offspring (90-92% of control value) and was slightly (n.s.) or significantly decreased in the high-concentration male offspring (92-94% of control value). Mean body weight gain was significantly decreased in the high-concentration females (82-89% of control value) during PNDs 7-11 and 13-17 and PND 4-21 (91% of controls). Mean weight gain was significantly decreased in the high-concentration males (83% of control value) during the PND 13-17 interval. The mid-concentration males and females also had reduced body weight gain (87-88% of control value) during the PND 13-17 interval.

   Selected mean post-weaning body weight and body weight gain data on offspring assigned to the FOB are presented in Table 6. Absolute body weight of the high-concentration group was significantly less than that of controls through PND 56 for males (92-95% of controls) and PND 42 for females (91-95% of controls). Body weight for the low-concentration females was significantly less than that of controls on PNDs 28 and 35. Thereafter until study termination on PND 72, body weight was comparable between the treated and control groups in both sexes. Weight gain by the high-concentration males and females was significantly less than that of the controls during the PND 28-35 interval. Body weight gain was similar between the treated and control groups for all intervals after PND 35.

TABLE 5. Selected mean (±SD) pup body weight and body weight gain				
PND	Exposure concentration (ppm)			
	0	5	25	50
Males				
Body weight (g)				
1	7.5 ± 0.62	7.5 ± 0.64	7.6 ± 0.51	7.3 ± 0.68
4 (pre-cull)	10.6 ± 0.99	11.1 ± 1.07	11.0 ± 1.10	10.5 ± 1.07
7	16.2 ± 1.46	16.7 ± 1.29	16.6 ± 1.68	15.6 ± 1.88
11	25.3 ± 2.33	25.3 ± 2.11	25.5 ± 2.81	24.0 ± 2.58
13	30.0 ± 2.65	29.7 ± 2.53	29.9 ± 3.20	28.3 ± 2.81
17	40.0 ± 3.51	38.8 ± 3.31	38.7 ± 4.11	36.6** ± 3.41 (92)[a]
21	53.3 ± 4.88	51.0 ± 4.86	51.9 ± 5.65	49.8 ± 5.10
Body weight gain (g)				
1-4	3.1 ± 0.55	3.6 ± 0.59	3.5 ± 0.69	3.2 ± 0.56
4-7	5.6 ± 0.92	5.6 ± 0.43	5.5 ± 0.85	5.1 ± 0.97
7-11	9.1 ± 1.21	8.7 ± 1.09	8.9 ± 1.38	8.5 ± 0.97
11-13	4.8 ± 0.66	4.4 ± 0.99	4.5 ± 0.77	4.3 ± 0.68
13-17	10.0 ± 1.36	9.1 ± 1.40	8.8** ± 1.17 (88)	8.3** ± 0.96 (83)
17-21	13.3 ± 2.50	12.2 ± 2.59	13.3 ± 2.53	13.2 ± 2.40
4-21	42.7 ± 4.30	39.9 ± 4.25	40.9 ± 4.83	39.3 ± 4.25
Females				
Body weight (g)				
1	7.1 ± 0.62	7.2 ± 0.62	7.1 ± 0.50	6.9 ± 0.59
4 (pre-cull)	10.2 ± 1.05	10.6 ± 1.08	10.6 ± 1.01	10.0 ± 0.93
7	15.7 ± 1.42	15.8 ± 1.63	15.8 ± 1.63	15.1 ± 1.55
11	24.9 ± 2.33	24.2 ± 2.55	24.3 ± 2.71	23.3 ± 2.20
13	29.7 ± 2.58	28.5 ± 2.84	28.7 ± 3.37	27.4* ± 2.24 (92)
17	39.4 ± 3.03	37.2 ± 3.96	37.2 ± 4.08	35.4** ± 2.80 (90)
21	51.9 ± 4.39	48.7 ± 5.55	49.5 ± 5.49	47.9* ± 3.95 (92)
Body weight gain (g)				
1-4	3.1 ± 0.59	3.5 ± 0.59	3.5 ± 0.65	3.2 ± 0.48
4-7	5.6 ± 0.69	5.2 ± 0.86	5.3 ± 0.82	5.0 ± 0.79
7-11	9.2 ± 1.23	8.4* ± 1.14	8.5 ± 1.37	8.2* ± 0.93 (89)
11-13	4.8 ± 0.67	4.3 ± 1.00	4.4 ± 0.95	4.1 ± 0.69
13-17	9.7 ± 1.15	8.7* ± 1.59 (90)	8.4** ± 1.09 (87)	8.0** ± 0.98 (82)
17-21	12.5 ± 2.44	11.5 ± 2.26	12.4 ± 2.46	12.5 ± 1.97
4-21	41.8 ± 3.72	38.1** ± 4.90 (91)	39.0 ± 4.78	37.9** ± 3.21 (91)

Data taken from Tables 24 and 25, pp. 241-243 and 244-246, respectively, MRID 46665001.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 6. Selected mean (±SD) post-weaning pup body weight and body weight gain				
PND	Exposure concentration (ppm)			
	0	5	25	50
Males				
Body weight (g)				
28	94.6 ± 7.28	90.0 ± 7.46	93.9 ± 8.43	87.5* ± 9.18 (92)[a]
35	159.5 ± 11.05	152.7 ± 12.37	158.9 ± 11.96	147.4** ± 13.28 (92)
49	289.9 ± 17.56	279.5 ± 18.56	290.3 ± 18.14	273.2* ± 21.54 (94)
72	439.7 ± 30.54	431.0 ± 27.13	443.2 ± 23.83	425.8 ± 25.94
Body weight gain (g)				
28-35	64.9 ± 4.93	62.7 ± 5.68	64.9 ± 4.90	59.9** ± 5.27 (92)
42-49	63.2 ± 6.40	61.5 ± 5.03	63.7 ± 4.92	60.9 ± 6.32
70-72	10.9 ± 3.21	10.0 ± 3.67	10.2 ± 2.41	9.3 ± 4.21
28-72	345.1 ± 26.40	341.0 ± 23.20	349.3 ± 20.16	338.3 ± 19.65
Females				
Body weight (g)				
28	88.3 ± 5.87	80.4** ± 9.04 (91)	85.5 ± 7.59	81.4** ± 5.54 (92)
35	141.0 ± 10.45	131.6** ± 9.97 (93)	135.8 ± 9.28	128.8** ± 9.13 (91)
49	203.3 ± 17.13	195.4 ± 13.25	199.5 ± 13.77	194.5 ± 12.59
72	267.6 ± 24.03	263.7 ± 18.28	263.5 ± 16.84	260.0 ± 16.84
Body weight gain (g)				
28-35	52.7 ± 5.48	51.2 ± 3.20	50.3 ± 3.58	47.4** ± 5.74
42-49	26.1 ± 6.54	26.0 ± 5.90	25.6 ± 5.32	26.8 ± 6.62
70-72	1.3 ± 7.03	4.1 ± 5.00	3.9 ± 5.01	3.5 ± 4.71
28-72	179.3 ± 20.35	183.3 ± 17.99	178.1 ± 12.55	178.7 ± 14.44

Data taken from Tables 32-35, pp. 255-262, MRID 46665001.
N = 20/sex/group
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

3. Developmental landmarks:

a. Sexual maturation: Age and body weight at sexual maturation are given in Table 7. The average age of onset of preputial separation in males was significantly delayed by 1.4 days in the high-concentration group compared with the controls. The average age of onset of vaginal opening was in high-concentration females was significantly delayed by 1.6 days compared with the controls. Vaginal opening was also significantly delayed in the low concentration females. Body weight in the treated males and females was similar to that of the control group at the time of acquisition.

b. Developmental landmarks: Other endpoints of offspring development (eye opening, pinna unfolding, hair growth, etc.) were not monitored in this study.

TABLE 7. Mean (±SD) age and body weight at sexual maturation				
Parameter	Exposure concentration (ppm)			
	0	5	25	50
N (M/F)	20/20	20/20	20/20	20/20
Preputial separation				
mean age (days)	43.8 ± 1.31	44.6 ± 1.60	43.8 ± 1.68	45.2* ± 1.75
mean body weight (g)	243.3 ± 17.24	240.9 ± 14.71	242.0 ± 13.31	240.2 ± 18.57
Vaginal opening				
mean age (days)	32.3 ± 1.03	33.5* ± 1.46	32.8 ± 1.35	33.9** ± 1.61
mean body weight (g)	120.6 ± 8.78	120.9 ± 11.01	118.8 ± 8.46	119.2 ± 9.54

Data taken from Tables 28 and 29, pp. 249 and 250, respectively, MRID 46665001.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

4. Behavioral assessment:

a. Functional observational battery: No treatment-related FOB changes were observed in males or females on any testing day. Statistically significant findings included 3/20 high-concentration males with crusty deposits on the nose (vs 0/20 controls) on PND 60, increased grooming counts for high-concentration males on PND 21, decreased grooming counts for high-concentration males and females on PND 45 and for males on PND 60, and a decreased reaction to tail pinch in mid- and high-concentration males on PND 21.

b. Motor/locomotor activity: Total motor and ambulatory activity data are presented in Table 8. Interval data for total counts are presented in Tables 9 (males) and 10 (females); habituation was evident in all groups by PND 17. No significant difference in total activity or ambulatory activity was found between the treated and control groups on any testing day. High-concentration males and females and mid-concentration females had slightly reduced total and ambulatory activities on PND 21 due to reduced activity throughout the testing interval.

TABLE 8. Mean (±SD) total and ambulatory motor activity counts

Test day	Exposure concentration (ppm)			
	0	5	25	50
Males				
PND 13				
Total	656 ± 465.5	725 ± 531.1	750 ± 664.2	904 ± 681.8
Ambulatory	190 ± 218.2	266 ± 349.4	297 ± 406.5	377 ± 432.7
PND 17				
Total	491 ± 283.2	489 ± 202.8	502 ± 323.8	518 ± 608.6
Ambulatory	158 ± 119.5	147 ± 85.1	153 ± 123.5	160 ± 268.0
PND 21				
Total	821 ± 423.9	670 ± 352.3	608 ± 278.3	494 ± 199.8 (60)[a]
Ambulatory	272 ± 156.9	213 ± 127.8	187 ± 136.1	148 ± 66.7 (54)
PND 61				
Total	2250 ± 526.1	2245 ± 527.8	2185 ± 508.7	2317 ± 667.3
Ambulatory	778 ± 241.0	754 ± 244.2	729 ± 211.7	809 ± 269.4
Females				
PND 13				
Total	639 ± 417.7	707 ± 491.1	690 ± 538.1	813 ± 593.4
Ambulatory	207 ± 265.4	238 ± 284.1	215 ± 314.8	311 ± 354.2
PND 17				
Total	619 ± 381.7	556 ± 394.5	555 ± 274.1	539 ± 315.1
Ambulatory	212 ± 157.2	179 ± 148.4	172 ± 111.9	178 ± 132.7
PND 21				
Total	779 ± 351.1	744 ± 332.5	594 ± 256.6 (76)	490 ± 248.7 (63)
Ambulatory	251 ± 137.2	226 ± 87.6	170 ± 86.2 (68)	150 ± 94.8 (60)
PND 61				
Total	2089 ± 458.6	1951 ± 558.6	2092 ± 675.1	1954 ± 461.2
Ambulatory	821 ± 223.5	783 ± 241.9	818 ± 292.4	778 ± 168.1

Data taken from Table 40, pp. 349-378, MRID 46665001.
N=20/sex/group.
[a]Number in parentheses is percent of control; calculated by reviewer.

TABLE 9. Mean (± SD) sub-session motor activity count for males				
Interval (min)	Exposure concentration (ppm)			
	0	5	25	50
PND 13				
0-15	276.40 ± 228.637	307.85 ± 210.963	230.60 ± 173.132	278.15 ± 187.474
16-30	146.65 ± 194.861	160.00 ± 179.907	203.85 ± 212.567	253.25 ± 271.614
31-45	105.10 ± 70.006	140.95 ± 179.406	154.50 ± 260.771	180.05 ± 211.119
46-60	127.75 ± 155.438	116.00 ± 125.404	161.25 ± 196.486	192.90 ± 237.941
PND 17				
0-15	337.50 ± 175.975	375.65 ± 169.044	326.25 ± 163.468	335.85 ± 205.139
16-30	61.00 ± 103.870	40.25 ± 62.230	46.75 ± 91.895	74.45 ± 205.785
31-45	46.10 ± 83.201	36.90 ± 84.019	71.65 ± 140.652	61.90 ± 142.084
46-60	46.45 ± 72.629	36.45 ± 50.590	57.25 ± 85.312	45.50 ± 98.114
PND 21				
0-15	556.25 ± 124.332	495.00 ± 190.86	488.45 ± 231.679	377.90 ± 137.817(68)[a]
16-30	121.70 ± 151.176	96.15 ± 103.382	74.65 ± 77.443	71.85 ± 78.993 (59)
31-45	71.75 ± 107.594	47.25 ± 80.847	24.85 ± 38.257	28.10 ± 28.505 (39)
46-60	71.75 ± 122.970	32.00 ± 71.475	19.75 ± 17.702	16.60 ± 18.588 (23)
PND 61				
0-15	1093.70 ± 218.648	1081.05 ± 196.537	1015.55 ± 170.332	1030.85 ± 163.559
16-30	554.75 ± 177.225	524.85 ± 174.032	501.70 ± 125.936	542.75 ± 206.607
31-45	327.15 ± 220.422	326.60 ± 175.599	367.40 ± 178.801	445.95 ± 233.173
46-60	274.70 ± 212.221	312.55 ± 219.667	299.95 ± 203.902	297.50 ± 216.45

Data taken from Table 40, pp. 350-359, MRID 46665001.
N = 20.
[a]Number in parentheses is percent of control, calculated by reviewer.

TABLE 10. Mean (± SD) sub-session motor activity count for females				
Interval (min)	Exposure concentration (ppm)			
	0	5	25	50
PND 13				
0-15	284.40 ± 179.461	245.80 ± 191.196	267.50 ± 120.896	256.20 ± 179.179
16-30	145.20 ± 114.159	178.10 ± 196.426	192.75 ± 199.676	182.60 ± 198.552
31-45	114.70 ± 149.174	179.35 ± 168.082	97.800 ± 199.668	189.45 ± 177.209
46-60	94.600 ± 125.277	103.40 ± 100.331	131.45 ± 145.518	184.95 ± 254.079
PND 17				
0-15	423.80 ± 153.534	371.05 ± 161.947	369.85 ± 167.369	398.40 ± 227.104
16-30	89.50 ± 162.106	68.85 ± 115.541	65.25 ± 107.664	61.60 ± 138.226
31-45	59.30 ± 132.380	48.15 ± 98.247	70.55 ± 110.900	30.55 ± 49.698
46-60	46.10 ± 68.537	67.90 ± 96.257	49.35 ± 96.977	48.10 ± 69.289
PND 21				
0-15	521.50 ± 152.66	480.55 ± 131.848	429.25 ± 144.305 (82)[a]	364.05 ± 141.138 (70)
16-30	149.55 ± 134.099	117.30 ± 117.862	88.25 ± 105.944 (59)	68.15 ± 90.808 (46)
31-45	49.25 ± 65.539	94.95 ± 128.924	31.50 ± 32.067 (64)	35.90 ± 78.610 (73)
46-60	58.95 ± 111.646	50.90 ± 69.549	45.40 ± 91.294 (77)	22.25 ± 44.701 (38)
PND 61				
0-15	1001.00 ± 230.998	969.35 ± 202.23	891.80 ± 155.018	940.35 ± 110.451
16-30	517.25 ± 181.571	481.95 ± 167.105	529.10 ± 226.277	452.60 ± 121.279
31-45	334.20 ± 187.814	304.75 ± 235.158	336.45 ± 203.994	304.10 ± 211.624
46-60	236.50 ± 201.555	194.85 ± 182.085	335.10 ± 200.498	257.40 ± 194.163

Data taken from Table 40, pp. 351-360, MRID 46665001.
N = 20.
[a]Number in parentheses is percent of control, calculated by reviewer.

c. **Auditory startle reflex habituation:** Overall maximum response amplitude (V_{MAX}), latency to V_{MAX} (T_{MAX}) and average response amplitude (V_{AVE}) data in male and female rats are presented in Table 11. Mean interval data are presented in Table 12. No statistically significant differences between treated and control groups were observed. Habituation was somewhat apparent on PND 20 and clearly evident on PND 60.

TABLE 11. Mean (±SD) total acoustic startle peak amplitude (V_{MAX}), latency to peak (T_{MAX}) and average response amplitude (V_{AVE})

Exposure conc. (ppm)	Parameter	Males		Females	
		PND 20	PND 60	PND 20	PND 60
0	V_{MAX} (mv)	960.27 ± 495.825	670.31 ± 440.160	846.08 ± 370.715	428.39 ± 274.840
	T_{MAX} (msec)	125.18 ± 22.746	156.95 ± 24.310	115.51 ± 18.745	165.85 ± 23.080
	V_{AVE} (mv)	192.34 ± 98.944	127.59 ± 86.657	162.61 ± 62.038	69.765 ± 50.012
5	V_{MAX} (mv)	873.81 ± 386.150	623.07 ± 474.812	1010.71 ± 522.577	424.61 ± 238.109
	T_{MAX} (msec)	120.03 ± 13.512	162.56 ± 28.185	117.59 ± 14.832	161.48 ± 19.768
	V_{AVE} (mv)	169.50 ± 85.301	127.96 ± 106.289	193.78 ± 106.442	73.625 ± 44.297
25	V_{MAX} (mv)	970.42 ± 407.756	769.55 ± 633.614	906.25 ± 353.130	406.48 ± 352.792
	T_{MAX} (msec)	128.20 ± 18.537	155.98 ± 26.016	112.82 ± 9.986	174.14 ± 34.093
	V_{AVE} (mv)	198.90 ± 85.269	148.18 ± 123.725	174.63 ± 67.478	69.730 ± 64.089
50	V_{MAX} (mv)	796.80 ± 302.881	660.14 ± 602.389	838.26 ± 292.936	467.34 ± 374.187
	T_{MAX} (msec)	126.41 ± 23.873	150.31 ± 13.414	114.34 ± 11.605	162.96 ± 24.070
	V_{AVE} (mv)	157.60 ± 75.304	126.13 ± 122.957	161.95 ± 63.695	83.41 ± 75.918

Data taken from Table 41, pp. 400-407, MRID 46665001.
N = 20/sex/group

TABLE 12. Interval data for acoustic startle peak amplitude (V_{MAX}), latency to peak (T_{MAX}) and average response amplitude (V_{AVE})

Exposure conc. (ppm)	Parameter	Blocks 1-10	Blocks 11-20	Blocks 21-30	Blocks 31-40	Blocks 41-50
Males - PND 20						
0	V_{MAX} (mv)	247.88 ± 118.79	210.21 ± 114.52	175.89 ± 102.19	161.78 ± 98.20	164.52 ± 94.17
	T_{MAX} (msec)	27.59 ± 7.21	24.43 ± 6.01	24.43 ± 7.40	23.24 ± 4.08	25.50 ± 4.12
	V_{AVE} (mv)	50.46 ± 25.97	41.42 ± 22.24	34.95 ± 19.47	31.89 ± 19.68	33.63 ± 20.19
5	V_{MAX} (mv)	210.10 ± 90.29	185.03 ± 100.22	173.21 ± 79.04	160.56 ± 73.50	144.93 ± 80.44
	T_{MAX} (msec)	26.26 ± 4.77	22.96 ± 3.04	23.88 ± 4.56	24.37 ± 4.70	22.56 ± 2.67
	V_{AVE} (mv)	41.72 ± 18.45	34.94 ± 20.98	32.72 ± 17.54	31.49 ± 16.83	28.65 ± 17.56
25	V_{MAX} (mv)	231.85 ± 104.48	197.15 ± 95.61	197.56 ± 82.57	182.87 ± 78.90	161.00 ± 84.40
	T_{MAX} (msec)	28.87 ± 5.88	24.39 ± 4.05	24.55 ± 3.66	25.97 ± 5.22	24.42 ± 4.22
	V_{AVE} (mv)	48.85 ± 23.60	38.92 ± 19.34	39.84 ± 16.63	38.22 ± 16.55	33.09 ± 17.13
50	V_{MAX} (mv)	183.79 ± 77.16	168.62 ± 77.03	163.46 ± 63.68	144.30 ± 55.23	136.63 ± 59.55
	T_{MAX} (msec)	28.43 ± 6.01	24.44 ± 8.09	24.39 ± 6.08	24.56 ± 4.78	24.60 ± 3.91
	V_{AVE} (mv)	36.71 ± 17.95	32.60 ± 17.11	31.91 ± 16.12	28.29 ± 14.03	28.10 ± 15.62
Males - PND 60						
0	V_{MAX} (mv)	209.43 ± 115.59	127.61 ± 99.54	119.37 ± 86.95	95.61 ± 79.63	118.30 ± 123.85
	T_{MAX} (msec)	33.02 ± 6.71	32.08 ± 10.17	30.05 ± 7.44	31.98 ± 7.25	29.84 ± 10.79
	V_{AVE} (mv)	41.36 ± 23.80	24.22 ± 19.25	22.23 ± 16.92	17.23 ± 15.34	22.56 ± 24.53
5	V_{MAX} (mv)	180.49 ± 109.35	137.17 ± 116.26	103.35 ± 95.57	111.21 ± 119.01	90.87 ± 88.22
	T_{MAX} (msec)	31.11 ± 4.39	32.52 ± 8.08	32.12 ± 7.43	34.44 ± 9.94	32.37 ± 8.83
	V_{AVE} (mv)	39.48 ± 25.25	26.82 ± 23.60	21.24 ± 21.69	22.35 ± 26.81	18.08 ± 20.78
25	V_{MAX} (mv)	296.10 ± 309.91	184.33 ± 182.61	108.32 ± 84.71	86.40 ± 80.41	94.41 ± 85.99
	T_{MAX} (msec)	29.81 ± 4.94	32.09 ± 8.69	30.59 ± 7.28	31.68 ± 7.69	31.82 ± 8.37
	V_{AVE} (mv)	60.04 ± 61.00	35.48 ± 34.94	19.83 ± 16.48	15.60 ± 15.84	17.25 ± 17.28
50	V_{MAX} (mv)	175.07 ± 140.61	145.58 ± 137.25	126.83 ± 119.45	92.59 ± 96.44	120.09 ± 197.26
	T_{MAX} (msec)	29.01 ± 4.42	31.20 ± 7.00	29.86 ± 5.72	30.61 ± 4.79	29.65 ± 4.35
	V_{AVE} (mv)	36.07 ± 31.27	27.40 ± 28.78	23.80 ± 23.98	17.42 ± 20.37	21.46 ± 36.64
Females - PND 20						
0	V_{MAX} (mv)	205.51 ± 102.04	174.67 ± 83.09	157.63 ± 80.28	154.44 ± 76.51	153.84 ± 92.56
	T_{MAX} (msec)	24.42 ± 5.11	22.15 ± 3.83	23.02 ± 4.92	23.67 ± 5.28	22.27 ± 5.08
	V_{AVE} (mv)	39.27 ± 16.57	34.07 ± 14.91	30.46 ± 14.45	30.00 ± 15.08	28.82 ± 16.57
5	V_{MAX} (mv)	258.81 ± 125.37	216.61 ± 115.45	205.21 ± 106.37	176.40 ± 114.75	153.69 ± 115.00
	T_{MAX} (msec)	24.85 ± 3.81	23.61 ± 6.27	25.52 ± 3.40	23.53 ± 5.68	23.08 ± 3.96
	V_{AVE} (mv)	48.88 ± 23.15	40.84 ± 22.97	39.56 ± 22.92	34.77 ± 24.57	29.75 ± 23.18

TABLE 12. Interval data for acoustic startle peak amplitude (V_{MAX}), latency to peak (T_{MAX}) and average response amplitude (V_{AVE})

Exposure conc. (ppm)	Parameter	Blocks 1-10	Blocks 11-20	Blocks 21-30	Blocks 31-40	Blocks 41-50
25	V_{MAX} (mv)	238.89 ± 102.05	179.76 ± 67.68	167.82 ± 80.25	161.99 ± 85.33	157.79 ± 75.87
	T_{MAX} (msec)	24.09 ± 3.23	22.73 ± 2.99	21.99 ± 3.56	22.30 ± 4.18	21.73 ± 1.99
	V_{AVE} (mv)	45.81 ± 17.80	34.06 ± 12.94	32.79 ± 16.07	30.92 ± 16.10	31.06 ± 15.34
50	V_{MAX} (mv)	190.48 ± 66.04	168.88 ± 70.33	161.56 ± 63.49	156.95 ± 66.70	160.40 ± 59.24
	T_{MAX} (msec)	25.37 ± 4.72	22.70 ± 2.80	22.60 ± 4.00	21.79 ± 2.50	21.89 ± 2.78
	V_{AVE} (mv)	36.60 ± 15.43	31.68 ± 15.18	30.83 ± 13.49	30.30 ± 14.27	32.55 ± 13.00
Females - PND 60						
0	V_{MAX} (mv)	126.53 ± 83.84	101.98 ± 80.94	77.38 ± 78.25	60.60 ± 48.98	61.92 ± 39.87
	T_{MAX} (msec)	32.28 ± 5.83	31.12 ± 6.39	34.13 ± 6.06	34.15 ± 8.23	34.19 ± 6.45
	V_{AVE} (mv)	22.17 ± 17.20	15.79 ± 13.12	12.49 ± 14.08	9.78 ± 8.98	9.54 ± 6.16
5	V_{MAX} (mv)	118.21 ± 73.01	85.90 ± 73.31	63.73 ± 32.86	79.45 ± 59.96	77.34 ± 67.00
	T_{MAX} (msec)	34.85 ± 7.63	33.31 ± 7.76	31.82 ± 6.08	28.83 ± 6.18	32.68 ± 7.71
	V_{AVE} (mv)	21.92 ± 14.07	13.95 ± 13.47	11.49 ± 6.82	13.18 ± 11.38	13.10 ± 12.38
25	V_{MAX} (mv)	116.30 ± 83.12	73.98 ± 71.55	70.89 ± 74.25	66.04 ± 69.82	79.28 ± 77.43
	T_{MAX} (msec)	35.12 ± 7.93	34.10 ± 8.88	34.63 ± 9.10	35.26 ± 10.34	35.05 ± 9.33
	V_{AVE} (mv)	20.47 ± 15.13	12.46 ± 13.13	12.33 ± 14.58	11.29 ± 13.28	13.19 ± 13.24
50	V_{MAX} (mv)	135.89 ± 134.83	96.94 ± 93.19	79.95 ± 84.99	83.27 ± 67.49	72.30 ± 70.99
	T_{MAX} (msec)	31.70 ± 5.70	33.02 ± 6.73	36.82 ± 7.38	28.99 ± 7.14	32.44 ± 7.96
	V_{AVE} (mv)	26.33 ± 30.15	17.61 ± 20.01	13.18 ± 16.65	14.49 ± 13.38	11.80 ± 13.20

Data taken from Table 41, pp. 382-398, MRID 46665001.
N = 20/sex/group

d. Learning and memory testing:

Watermaze performance: The watermaze performance data for PNDs 26 and 62 are presented in Tables 13 and 14, respectively. No treatment-related effects on learning and memory were found at either testing interval. On both testing days, learning was evident for all groups as a decrease in both time and number of errors with successive trials through both paths of the maze. Memory was also demonstrated for all groups on both testing days as a decrease in both endpoints in trial 12 compared with trial 11.

TABLE 13. Water maze performance of offspring on PND 26 (mean±SD)

Day/Trial	Exposure concentration (ppm)			
	0	5	25	50
Males				
Day 1 - Swimming Ability Mean Time (sec)	8.58 ± 2.374	8.87 ± 1.558	8.63 ± 2.294	8.65 ± 1.944
Trial 1 (Day 2) - Path A Mean Time (sec) Mean No. Errors	65.57 ± 40.10 13.25 ± 8.87	80.90 ± 41.89 16.60 ± 8.72	61.94 ± 31.64 12.70 ± 7.44	83.80 ± 53.75 16.70 ± 10.65
Trial 2 (Day2) - Path A Mean Time (sec) Mean No. Errors	63.85 ± 45.67 12.50 ± 9.93	53.49 ± 34.95 10.40 ± 8.11	55.22 ± 30.51 12.20 ± 9.22	43.80 ± 31.96 8.40 ± 8.37
Trial 3 (Day 3) - Path A Mean Time (sec) Mean No. Errors	39.23 ± 35.04 8.10 ± 10.28	41.55 ± 24.15 8.85 ± 7.06	51.07 ± 40.61 10.85 ± 10.30	53.79 ± 50.66 11.15 ± 14.99
Trial 4 (Day 3) - Path A Mean Time (sec) Mean No. Errors	33.76 ± 24.01 6.30 ± 6.57	28.07 ± 13.10 4.95 ± 4.02	31.21 ± 27.61 5.95 ± 6.45	47.31 ± 36.80 10.30 ± 10.50
Trial 5 (Day 4) - Path B Mean Time (sec) Mean No. Errors	145.97 ± 52.53 29.15 ± 12.85	155.70 ± 34.88 33.30 ± 8.55	155.10 ± 46.40 33.79 ± 11.39	165.93 ± 35.92 35.75 ± 8.55
Trial 6 (Day 4) - Path B Mean Time (sec) Mean No. Errors	100.36 ± 70.00 20.95 ± 15.93	120.52 ± 60.75 23.70 ± 14.08	127.31 ± 61.96 23.35 ± 13.81	139.48 ± 49.22 26.65 ± 10.84
Trial 7 (Day 5) - Path B Mean Time (sec) Mean No. Errors	90.00 ± 62.84 17.25 ± 15.12	98.59 ± 56.16 19.35 ± 12.41	118.00 ± 69.19 23.00 ± 16.16	83.31 ± 59.40 15.70 ± 12.56
Trial 8 (Day 5) - Path B Mean Time (sec) Mean No. Errors	51.49 ± 55.45 8.00 ± 9.79	65.12 ± 59.19 11.75 ± 13.28	94.13 ± 66.09 19.50 ± 16.00	71.40 ± 66.73 10.95 ± 11.94
Trial 9 (Day 6) - Path B Mean Time (sec) Mean No. Errors	37.90 ± 43.24 5.30 ± 5.77	49.79 ± 50.02 7.75 ± 8.30	69.31 ± 55.87 13.40 ± 11.70	60.12 ± 71.84 9.60 ± 12.89
Trial 10 (Day 6) - Path B Mean Time (sec) Mean No. Errors	39.89 ± 52.76 6.65 ± 11.69	49.69 ± 47.33 8.55 ± 9.39	30.71 ± 19.52 5.05 ±4.26	42.23 ± 44.61 6.95 ± 7.66
Trial 11 (Day 7) - Path A (Probe) Mean Time (sec) Mean No. Errors	64.40 ± 38.30 15.50 ± 12.14	66.96 ± 37.88 16.00 ± 12.12	68.93 ± 38.55 16.70 ± 11.89	80.35 ± 42.16 19.90 ± 14.30
Trial 12 (Day 7) - Path A (Probe) Mean Time (sec) Mean No. Errors	53.60 ± 38.77 11.15 ± 9.05	42.06 ± 21.06 7.80 ± 4.92	43.39 ± 20.57 9.50 ± 7.47	51.77 ± 34.41 10.10 ± 8.28

TABLE 13. Water maze performance of offspring on PND 26 (mean±SD)

Day/Trial	Exposure concentration (ppm)			
	0	5	25	50
Females				
Day 1 - Swimming Ability Mean Time (sec)	7.49 ± 1.364	8.34 ± 1.889	8.33 ± 2.917	8.62 ± 1.708
Trial 1 (Day 2) - Path A Mean Time (sec) Mean No. Errors	81.98 ± 54.58 16.90 ± 11.36	91.00 ± 57.01 16.05 ± 10.49	74.15 ± 53.37 14.65 ± 10.28	75.34 ± 49.58 15.90 ± 10.54
Trial 2 (Day 2) - Path A Mean Time (sec) Mean No. Errors	60.74 ± 45.93 13.60 ± 11.89	78.69 ± 56.46 15.20 ± 11.72	53.72 ± 44.58 10.85 ± 10.30	66.75 ± 47.03 13.85 ± 10.98
Trial 3 (Day 3) - Path A Mean Time (sec) Mean No. Errors	34.39 ± 18.06 8.15 ± 6.91	74.48 ± 52.35 15.95 ± 11.89	49.20 ± 35.30 10.70 ± 8.71	49.75 ± 41.51 10.25 ± 9.78
Trial 4 (Day 3) - Path A Mean Time (sec) Mean No. Errors	35.05 ± 19.89 7.95 ± 7.20	48.48 ± 39.01 10.50 ± 9.34	32.93 ± 25.73 6.60 ± 7.49	34.39 ± 25.12 7.40 ± 6.82
Trial 5 (Day 4) - Path B Mean Time (sec) Mean No. Errors	127.03 ± 53.65 27.75 ± 14.13	144.54 ± 48.29 31.80 ± 11.86	139.54 ± 54.91 30.80 ± 13.75	145.44 ± 49.06 31.10 ± 12.56
Trial 6 (Day 4) - Path B Mean Time (sec) Mean No. Errors	98.10 ± 67.07 21.05 ± 16.30	109.43 ± 62.75 21.75 ± 13.90	94.77 ± 65.16 19.05 ± 14.65	120.83 ± 61.45 22.65 ± 13.88
Trial 7 (Day 5) - Path B Mean Time (sec) Mean No. Errors	106.75 ± 62.30 20.95 ± 12.88	88.33 ± 66.94 16.95 ± 13.79	81.51 ± 54.49 15.85 ± 12.49	103.84 ± 72.28 20.60 ± 16.14
Trial 8 (Day 5) - Path B Mean Time (sec) Mean No. Errors	102.20 ± 67.31 20.80 ± 15.62	80.14 ± 58.62 13.00 ± 11.41	50.23 ± 53.03 8.55 ± 11.77	70.17 ± 68.80 13.80 ± 16.56
Trial 9 (Day 6) - Path B Mean Time (sec) Mean No. Errors	71.83 ± 57.65 12.35 ± 11.41	77.76 ± 61.46 14.70 ± 12.86	50.54 ± 52.29 9.25 ± 12.42	71.72 ± 66.78 14.05 ± 14.54
Trial 10 (Day 6) - Path B Mean Time (sec) Mean No. Errors	75.98 ± 66.29 13.95 ± 14.71	51.38 ± 50.15 9.55 ± 13.04	39.40 ± 39.24 6.85 ± 7.73	47.75 ± 46.60 8.80 ± 10.69
Trial 11 (Day 7) - Path A (Probe) Mean Time (sec) Mean No. Errors	82.72 ± 48.68 19.00 ± 12.09	72.66 ± 29.53 16.60 ± 10.07	77.33 ± 43.06 18.55 ± 12.76	78.75 ± 46.90 18.40 ± 11.96
Trial 12 (Day 7) - Path A (Probe) Mean Time (sec) Mean No. Errors	59.98 ± 47.16 13.45 ± 13.96	46.50 ± 34.31 7.70 ± 7.37	39.46 ± 24.81 7.20 ± 5.79	57.84 ± 32.54 11.75 ± 8.53

Data taken from Table 42 and 43, pp. 408-409 and 410-445, respectively, MRID 46665001.
N=20

TABLE 14. Water maze performance of offspring on PND 62 (mean±SD)

Day/Trial	Exposure concentration (ppm)			
	0	5	25	50
Males				
Day 1 - Swimming Ability Mean Time (sec)	7.34 ± 1.905	7.45 ± 2.023	7.96 ± 2.838	8.36 ± 2.756
Trial 1 (Day 2) - Path A Mean Time (sec) Mean No. Errors	83.45 ± 53.94 14.95 ± 9.24	63.14 ± 39.42 13.65 ± 9.97	72.60 ± 55.64 13.15 ± 9.28	71.37 ± 48.58 13.20 ± 9.09
Trial 2 (Day2) - Path A Mean Time (sec) Mean No. Errors	57.53 ± 42.27 11.05 ± 7.56	57.15 ± 43.81 11.55 ± 10.23	71.55 ± 47.77 14.10 ± 8.89	47.93 ± 33.66 9.90 ± 7.13
Trial 3 (Day 3) - Path A Mean Time (sec) Mean No. Errors	33.24 ± 24.41 6.00 ± 4.90	43.02 ± 37.21 8.30 ± 7.41	31.00 ± 21.58 6.20 ± 4.89	33.55 ± 25.75 6.65 ± 5.64
Trial 4 (Day 3) - Path A Mean Time (sec) Mean No. Errors	24.73 ± 18.65 3.65 ± 5.05	21.96 ± 16.82 3.90 ± 4.98	26.26 ± 36.73 5.35 ± 9.81	25.13 ± 19.44 4.75 ± 5.05
Trial 5 (Day 4) - Path B Mean Time (sec) Mean No. Errors	128.11 ± 54.73 22.65 ± 12.17	152.53 ± 51.93 27.11 ± 11.23	136.40 ± 58.40 26.75 ± 11.50	144.58 ± 55.38 26.11 ± 11.25
Trial 6 (Day 4) - Path B Mean Time (sec) Mean No. Errors	79.17 ± 63.65 14.45 ± 12.49	95.87 ± 67.37 14.65 ± 10.68	93.85 ± 74.45 16.00 ± 13.29	95.02 ± 59.59 18.70 ± 13.81
Trial 7 (Day 5) - Path B Mean Time (sec) Mean No. Errors	97.16 ± 62.86 16.40 ± 11.60	65.07 ± 60.07 9.45 ± 9.39	85.70 ± 66.75 14.35 ± 10.80	59.76 ± 55.60 9.90 ± 10.13
Trial 8 (Day 5) - Path B Mean Time (sec) Mean No. Errors	63.91 ± 59.19 10.95 ± 10.89	34.07 ± 36.41 4.40 ± 3.47	66.22 ± 65.14 11.75 ± 13.54	36.28 ± 34.82 6.00 ± 7.99
Trial 9 (Day 6) - Path B Mean Time (sec) Mean No. Errors	52.55 ± 57.05 9.45 ± 11.44	32.65 ± 41.52 3.65 ± 5.34	38.17 ± 50.98 5.10 ± 9.36	28.30 ± 18.75 4.30 ± 4.84
Trial 10 (Day 6) - Path B Mean Time (sec) Mean No. Errors	30.30 ± 28.12 5.75 ± 8.42	28.40 ± 37.95 3.10 ± 4.48	43.94 ± 60.26 6.25 ± 10.42	22.21 ± 13.01 3.35 ± 3.94
Trial 11 (Day 7) -Path A (Probe) Mean Time (sec) Mean No. Errors	72.13 ± 47.15 13.65 ± 10.35	72.16 ± 51.19 13.40 ± 10.87	48.55 ± 26.37 9.70 ± 6.25	73.62 ± 41.63 14.15 ± 10.36
Trial 12 (Day 7) -Path A (Probe) Mean Time (sec) Mean No. Errors	28.89 ± 16.21 4.05 ± 3.07	47.86 ± 39.90 8.65 ± 9.04	38.55 ± 25.07 7.10 ± 7.40	52.79 ± 36.57 8.60 ± 7.78

TABLE 14. Water maze performance of offspring on PND 62 (mean±SD)				
Day/Trial	Exposure concentration (ppm)			
	0	5	25	50
Females				
Day 1 - Swimming Ability Mean Time (sec)	7.94 ± 4.197	8.07 ± 2.241	8.10 ± 2.306	7.38 ± 1.893
Trial 1 (Day 2) - Path A Mean Time (sec) Mean No. Errors	65.20 ± 37.91 13.55 ± 9.20	80.69 ± 63.91 16.35 ± 14.49	72.27 ± 44.95 14.15 ± 9.10	59.89 ± 29.57 12.65 ± 7.65
Trial 2 (Day 2) - Path A Mean Time (sec) Mean No. Errors	57.93 ± 48.26 11.95 ± 9.56	68.34 ± 36.25 14.50 ± 9.47	66.17 ± 47.19 14.10 ± 10.38	60.60 ± 42.96 12.60 ± 11.04
Trial 3 (Day 3) - Path A Mean Time (sec) Mean No. Errors	40.39 ± 38.04 6.85 ± 6.95	71.39 ± 52.90 13.05 ± 11.94	52.92 ± 45.15 10.90 ± 10.66	42.34 ± 38.27 7.80 ± 8.03
Trial 4 (Day 3) - Path A Mean Time (sec) Mean No. Errors	23.86 ± 17.37 3.10 ± 3.81	26.14 ± 12.80 3.75 ± 3.48	27.53 ± 15.96 5.15 ± 5.41	24.17 ± 20.65 3.50 ± 4.43
Trial 5 (Day 4) - Path B Mean Time (sec) Mean No. Errors	130.57 ± 52.03 25.95 ± 10.84	117.05 ± 54.97 21.65 ± 10.01	128.48 ± 57.04 27.21 ± 14.27	103.51 ± 64.87 21.20 ± 15.24
Trial 6 (Day 4) - Path B Mean Time (sec) Mean No. Errors	84.20 ± 65.91 14.35 ± 12.76	89.66 ± 72.95 14.10 ± 13.59	61.70 ± 58.30 11.50 ± 13.92	64.20 ± 51.04 11.75 ± 10.83
Trial 7 (Day 5) - Path B Mean Time (sec) Mean No. Errors	51.75 ± 47.26 9.05 ± 9.31	80.09 ± 61.82 12.45 ± 10.79	54.41 ± 60.04 9.55 ± 12.98	72.50 ± 60.52 13.65 ± 13.06
Trial 8 (Day 5) - Path B Mean Time (sec) Mean No. Errors	32.23 ± 26.60 4.80 ± 5.60	47.85 ± 44.97 7.10 ± 7.63	32.33 ± 28.68 5.00 ± 6.42	38.91 ± 41.34 5.60 ± 6.76
Trial 9 (Day 6) - Path B Mean Time (sec) Mean No. Errors	37.11 ± 39.51 5.47 ± 9.76	30.87 ± 21.41 3.95 ± 4.83	41.95 ± 43.26 8.70 ± 13.16	36.24 ± 38.60 5.25 ± 6.62
Trial 10 (Day 6) - Path B Mean Time (sec) Mean No. Errors	34.98 ± 36.62 4.15 ± 5.30	32.23 ± 34.33 5.75 ± 10.46	38.88 ± 32.25 7.00 ± 8.34	34.74 ± 33.13 5.35 ± 7.10
Trial 11 (Day 7) -Path A (Probe) Mean Time (sec) Mean No. Errors	85.91 ± 57.80 15.05 ± 11.96	56.50 ± 34.23 11.40 ± 9.33	50.45 ± 28.95 10.65 ± 7.61	56.38 ± 34.48 12.35 ± 9.90
Trial 12 (Day 7) -Path A (Probe) Mean Time (sec) Mean No. Errors	42.00 ± 26.32 6.60 ± 5.97	44.95 ± 44.60 6.95 ± 10.15	32.60 ± 19.26 4.60 ± 5.46	34.82 ± 19.12 6.25 ± 6.70

Data taken from Tables 42 and 43, pp. 408-409 and 410-445, respectively, MRID 46665001.
N=20

5. Postmortem results:

a. **Brain weight:** Mean absolute brain weight data are presented in Table 15. No significant differences were observed between treated and control groups on either PND 21 or 72.

TABLE 15. Brain weight data (mean g±SD) from offspring

Sex and age	Exposure concentration (ppm)			
	0	5	25	50
Males - PND 21	1.6840 ± 0.08513	1.6719 ± 0.08025	1.6532 ± 0.05518	1.6225 ± 0.07216
Females - PND 21	1.6359 ± 0.06987	1.6063 ± 0.08742	1.6012 ± 0.12703	1.5512 ± 0.06649
Males - PND 72	2.23 ± 0.102	2.26 ± 0.125	2.27 ± 0.122	2.18 ± 0.099
Females - PND 72	2.06 ± 0.110	2.06 ± 0.095	2.04 ± 0.130	2.03 ± 0.105

Data taken from Tables 45 and 50, pp. 448-449 and 462-463, respectively, MRID 46665001.
N=15

b. **Macroscopic examination:** No remarkable changes were seen in pups found dead during lactation, pups selected for necropsy on PND 21, or pups selected for neuropathology on PNDs 21 and 72.

c. **Neuropathology**:

1) **Microscopic examination:** No treatment-related microscopic lesions were observed on PNDs 21 or 72. Degeneration of several nerves and retinal dysplasia were seen at low incidence in both control and high-concentration animals on PND 72.

2) **Brain Morphometry:** Brain length and width measurements are given in Table 16 and data from morphometric measurements are presented in Table 17. On PND 21, high-concentration males had significantly smaller brain width and high-concentration females had significantly smaller length of the ventral limb of the dentate hilus compared to the respective control values. On PND 72, high-concentration males had significantly larger measurement for the base of lobule 9. No other differences in any measurement were noted between treated and control groups for either sex at any time point.

TABLE 16. Brain length and width measurements (mean mm±SD) of rats on PNDs 21 and 72

Endpoint	Exposure concentration (ppm)			
	0	5	25	50
PND 21				
Males				
length	18.6 ± 0.51	18.6 ± 0.42	18.4 ± 0.39	18.4 ± 0.45
width	15.1 ± 0.28	15.0 ± 0.37	14.8 ± 0.43	14.7* ± 0.32
Females				
length	18.5 ± 0.44	18.4 ± 0.41	18.3 ± 0.63	18.3 ± 0.35
width	14.7 ± 0.51	14.9 ± 0.45	14.7 ± 0.41	14.7 ± 0.33
PND 72				
Males				
length	21.0 ± 0.45	21.3 ± 0.30	21.3 ± 0.44	21.1 ± 0.43
width	15.8 ± 0.25	15.8 ± 0.29	16.0 ± 0.24	15.8 ± 0.36
Females				
length	20.8 ± 0.37	20.6 ± 0.25	20.6 ± 0.31	20.5 ± 0.37
width	15.5 ± 0.27	15.5 ± 0.22	15.4 ± 0.24	15.5 ± 0.25

Data taken from Tables 45 and 50, pp. 448-449 and 462-463, respectively, MRID 46665001.
Significantly different from control:*$p \leq 0.05$.

TABLE 17. Brain morphometric measurements (mean cm±SD) from rat offspring on PNDs 21 and 72				
Parameter	Exposure concentration (ppm)			
	0	50	0	50
	Males		Females	
PND 21				
Height hemisphere	0.6677 ± 0.01985	0.6769 ± 0.03843	0.6341 ± 0.09895	0.6277 ± 0.10364
Vertical thickness cortex	0.1668 ± 0.00818	0.1657 ± 0.01074	0.1611 ± 0.01167	0.1590 ± 0.00481
Radial thickness cortex	0.1336 ± 0.00852	0.1368 ± 0.00804	0.1319 ± 0.00602	0.1332 ± 0.00673
Vertical height between hippocampal pyramidal neuron layers	0.0758 ± 0.00477	0.0771 ± 0.00498	0.0752 ± 0.00589	0.0766 ± 0.00670
Vertical height of dentate hilus	0.0392 ± 0.00265	0.0410 ± 0.00361	0.0404 ± 0.00181	0.0398 ± 0.00372
Length ventral limb dentate hilus	0.1210 ± 0.01019	0.1222 ± 0.01166	0.1233 ± 0.00885	0.1133* ± 0.00782
Vertical thickness of pons	0.2452 ± 0.02527	0.2538 ± 0.02388	0.2539 ± 0.02908	0.2548 ± 0.03151
Base of lobule 9	0.0591 ± 0.00395	0.0606 ± 0.00273	0.0607 ± 0.00419	0.0577 ± 0.00529
PND 72				
Height hemisphere	0.6366 ± 0.02618	0.6353 ± 0.03795	0.6189 ± 0.04171	0.6400 ± 0.02983
Vertical thickness cortex	0.1693 ± 0.00568	0.1595 ± 0.01694	0.1651 ± 0.01068	0.1662 ± 0.00888
Radial thickness cortex	0.1404 ± 0.00827	0.1371 ± 0.00856	0.1411 ± 0.00978	0.1389 ± 0.00829
Vertical height between hippocampal pyramidal neuron layers	0.0921 ± 0.00563	0.0961 ± 0.00719	0.0883 ± 0.00582	0.0883 ± 0.00578
Vertical height of dentate hilus	0.0485 ± 0.00363	0.0502 ± 0.00287	0.0462 ± 0.00304	0.0469 ± 0.00206
Length ventral limb dentate hilus	0.1388 ± 0.00900	0.1339 ± 0.01621	0.1290 ± 0.01103	0.1362 ± 0.00930
Vertical thickness of pons	0.2725 ± 0.02608	0.2648 ± 0.02976	0.2619 ± 0.02595	0.2621 ± 0.02387
Base of lobule 9	0.0664 ± 0.00520	0.0718* ± 0.00602	0.0664 ± 0.00699	0.0635 ± 0.00538

Data taken from Table 48 and 53, pp. 456-459 and 478-481, respectively, MRID 46665001.
N = 10
Significantly different from control: *p ≤ 0.05.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that no maternal toxicity or effects on reproduction were observed at any exposure level. Offspring toxicity was evident as reduced body weight gain resulting in reduced body weight in the 50 ppm group. The study author concluded that the mean day of acquisition of balanopreputial separation or vaginal patency and the mean body weight at attainment were not affected by treatment. Developmental neurotoxicity was seen as reduced motor activity in males and females at 50 ppm and in females at 25 ppm on PND 21. The maternal systemic and reproductive toxicity NOAEL is 50 ppm; the offspring developmental toxicity NOAEL is 25 ppm; and the offspring developmental neurotoxicity NOAEL is 5 ppm.

B. REVIEWER COMMENTS: Sacrifice of one control female with dystocia was considered incidental to test article exposure. No evidence of systemic or neurotoxicity was seen in maternal animals at any time during the study. FOB parameters, body weight, and reproductive performance were similar between the treated and control animals and maternal necropsy was unremarkable.

In the low-concentration group, a number of offspring parameters were significantly different from those of the control group. Fewer pups born resulting in smaller mean live litter size corresponded with fewer implantation sites for the dams. Slightly reduced body weight and body weight gain by the female offspring during lactation and post-weaning was probably the reason for the delay in sexual maturation observed for these pups. It is unclear why these endpoints were affected in the low-concentration group and a concentration-response did not occur. Therefore, effects in the low-concentration group are considered incidental to treatment.

Offspring survival was similar between all groups and no clinical signs of toxicity were observed during lactation. During lactation, body weight gain was reduced in the mid- and high-concentration males and females during days 13-17. The magnitude of lower weight gain was sufficient to cause reduced absolute body weight only in the high-concentration pups. The effect on absolute body weight extended into the post-weaning period. The transient decrease in weight gain for the mid- and high-concentration groups occurred at a critical time when the pups were transitioning to solid food and is considered treatment-related. The reviewer disagrees with the study author and thinks that the delay in sexual maturation observed in the high-concentration males and females was a result of lower body weight. Recovery of body weight by the high-concentration group to control levels occurred at approximately the same time as sexual maturation for both males and females.

Limited evidence of developmental neurotoxicity was seen as reduced motor activity on PND 21 in mid-concentration females and high-concentration males and females. Although no statistically significant differences occurred, both total and ambulatory activities were reduced throughout the testing interval. Lack of statistical significance was due to the high variability in the data. The effect on motor activity occurred on the last day of exposure, when any potential cumulative effect would have been maximized. No long-term effects were noted since activity was similar between the treated and control groups on PND 61. FOB parameters and learning and memory were not affected by treatment.

At PND 21 necropsy, the high-concentration males had a significant decrease in brain width compared to that of the controls. Although considered to be treatment-related, the decrease in brain width did not correspond to any differences in brain weight, other morphometric measurements, or histopathological findings and was not persistent over time.

The maternal systemic and neurotoxicity LOAEL for methyl bromide in rats is not identified and the maternal NOAEL is ≥50 ppm.

The offspring systemic and neurotoxicity LOAEL for methyl bromide in rats is 25 ppm based on decreased body weight gain in males and females and decreased motor activity in females. The offspring NOAEL is 5 ppm.

C. **STUDY DEFICIENCIES:** No major deficiencies were found in the conduct of this study. It is noted that adequate positive control studies have been submitted to demonstrate proficiency of the testing facility only for FOB, motor activity, and auditory startle tests in young adult rats. Adequate positive control data have not been submitted for learning and memory or neuropathology and morphometrics.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Exposure method	Exp. range (ppm)	Exposures tested (ppm)	NOAEL (ppm)	LOAEL (ppm)	Target organ(s)	Comments
053201	46665001	Dev Neurotox	Rat	GD 6-20 and LD 5-20	Inhal.	Whole-body	0-50	0, 5, 25, 50	≥50	not identified	none	Maternal systemic/ neurotoxic.
053201	46665001	Dev Neurotox	Rat	LD 5-20	Inhal.	Whole-body	0-50	0, 5, 25, 50	5	25	body weight gain during lactation in males and females, motor activity in females	Offspring systemic/ neurotoxic